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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/11961 A1

(51) International Patent Classification[7]: A01N 43/22 // (A01N 43/22, 25:34, 25:00)

(21) International Application Number: PCT/US00/17869

(22) International Filing Date: 31 July 2000 (31.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/148,524 12 August 1999 (12.08.1999) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/11961 A1

(54) Title: CONTROL OF ECTOPARASITES USING SPINOSYNS

(57) Abstract: The invention provides formulations for controlling insects/pests in small ruminants, such as camellids, sheep, and goats, the formulations comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent, and methods of controlling these insect pests comprising topically applying these formulations to a ruminant.

CONTROL OF ECTOPARASITES USING SPINOSYNS

This invention relates to A83543 compounds including analogues and derivatives thereof, and in particular the application of such compounds as insecticides in controlling lice, flies and other ectoparasites and related arthropod pests which infest sheep, goats, other small ruminant species and camelids (including alpacas, llamas, vacuna). Historically, the greatest damage to domestic animals has been caused, and continues to be caused, by pests such as insects. Insects particularly represent a cause for concern as they are the most numerous of all living organisms and constitute approximately 72% of all animal species. Approximately 1% of insects are considered pests in that they attack humans and/or domestic animals, transmit human, animal and plant diseases, destroy objects and structures and compete for food and other necessities.

The losses resulting from insect-caused human and animal diseases are enormous. In fact, insects are considered to be the carriers of more than 250 viruses which are pathogens of humans and higher animals. The numbers of human deaths caused by mosquito-transmitted diseases such as malaria and lymphatic filariasis are huge. Flies also transmit human- and animal-related diseases such as trachoma, trypanosomiasis and river blindness. Other human and animal diseases are transmitted by fleas and lice.

To date, the primary method for controlling insects, particularly in respect of domestic animals and crops, has been by the application of synthetic chemical insecticide compositions. It is estimated that there are at least 35,000 formulated insecticide products worldwide with chemicals as the active ingredients. Such

insecticide products include antimicrobials, larvicides, insecticides, animal dips, avicides and disinfectants.

The extensive use of chemical insecticides since the 1940s has resulted in a large number of problems, including widespread insect resistance, emergence of secondary pests, hazards to human and animal health, as well as detrimental effects on fish and birds and environmental pollution.

Many insect species have developed resistance to the action of specific insecticides so as to necessitate changes in control practices. There is an ever widening pool of insect pests which are developing multiple resistance. The resistant genes persist in insect genomes, precluding successful reuse of an insecticide to control an insect population with resistant genes. Thus, there are increasing reasons to develop new insecticides due to insect resistance.

Plant-derived insecticides such as nicotine, rotenone, veratrine and pyrethrum have also been used as natural contact insecticides to kill insect pests. However, the disadvantage with some plant-derived insecticides is their toxicity to humans, animals and fish.

Insecticide residues are also seen as a major problems resulting from chemical usage. With the exception of microbial insecticides, nearly all insecticides result in residues of various chemicals and their degradation products or metabolites which may be present in detectable amounts (ppb to ppm) in food despite food processing. With increasing use of insecticides, the nature and magnitude of such persisting residues have assumed great significance in public health. Potential environmental risks include threats to ground water or surface water (lakes, dams, rivers, streams). The persistence, mobility and potential for accumulation of an

insecticide and its primary degradates must be considered in order to assess the environmental impact of the use of an insecticide. Concern about the fate of bio-active chemicals introduced into the environment has led to strict regulations on release of insecticide waste into water, as well as for proper disposal of containers and waste from use of insecticides. Other factors such as corrosiveness, explosiveness and flammability of the insecticide must also be considered.

Some of the currently used products, such as pyrethroids and organophosphates (OP's), are potentially toxic to sheep, goats, other small ruminants and camelids, to the human operator who applies the treatment, and to the environment, such as when run-off or the effluent from wool processing plants is discharged into water ways. Thus, a potentially broad spectrum of toxicities exists with respect to insecticides used in the treatment or eradication of ectoparasites.

It is acknowledged that the use of insecticides is essential for high yield production of quality wool and to protect sheep from attack by ectoparasites (mainly lice and blowfly) through the growing season. Inevitably, however, the use of insecticides leaves some residue in the wool at shearing time. Accordingly, wool residues resulting from currently used insecticides are of major concern. Particularly, use of insecticides in late season treatments can lead to the occurrence of residues in the fleece at shearing. Late season backline and jetting treatments have been studied to calculate the dissipation of the active agents and to assess the spread of insecticide through the fleece. Insecticide formulations using representative members of organophosphate, synthetic pyrethroid or insect growth regulator insecticides commonly used to control lice and flies on sheep often leave high levels of wool residues when applied as late season backline treatments or as late season jetting

applications. The residues resulting from such standard, currently used products have been the cause of concern in the wool industry for some time. Further, the use of these products can also result in environmental discharge limits being exceeded.

The two processing issues with the highest priority relate to 1) the acceptability of lanolin products that are made from wool wax that is recovered during scouring, and 2) the residues that associate with the wool wax that is discharged from the scour with the processing wastes. Insecticides in the wool wax that is recovered when the wool is scoured may partially survive the lanolin refining process unless special steps are taken. For example, several years ago, diazinon was found in some samples of pharmaceutical lanolin and was considered a potential health hazard to infants.

Aqueous wool scouring produces high volumes of effluent containing emulsified wool wax, dirt and insecticides. While this effluent is treated before discharge to the environment, the impact on the environment depends on the extent of effluent treatment and dilution after scouring and on the location of the scour. If present in high enough concentrations in the effluent, insecticide residues can kill beneficial aquatic fauna which help the process of sewage degradation. Once released from sewage systems into streams or rivers, these chemical residues can interfere with the waterway food chain.

Currently, Australia and other countries export a substantial amount of their wool in a greasy form. That wool may be scoured and processed in many different countries and environmental locations, leaving international environmental issues as a major concern when considering wool residues. European environmental authorities have commenced the introduction of tough new guidelines for discharge of

chemicals into waterways. These regulations mean that both wool processors and growers have to change their practices if European customers are to continue to process wool. The total concentration of organophosphates and synthetic pyrethroids in the Australian wool clip in 1994-1995 averaged 10 mg/kg (ppm) on greasy wool. While this amount has been reduced over the last two years, Europe has indicated that chemical residue levels on greasy wool will need to be reduced to 1-2 mg/kg over the next 9 years in order to comply with new EC standards. For some pesticides, residue levels must be even lower.

It is to be noted that the Australian wool industry has set a goal to reduce the level of insecticide residues in the wool clip over the next few years, and therefore new safer insecticides are required. Other wool growing countries in the International wool network are also moving to reduce residues of all insecticides. Europe, Canada, Japan and New Zealand all have eco-labelling schemes of wool and textile products which focus on absence of toxic substances, etc. In order to accomplish these goals, new, safer, low residue insecticides will be required.

Tissue residues are also a major concern when considering use of insecticides on domestic animals. Application of an insecticide to a domestic animal may result in toxic chemical residues being collected in the meat/tissue of the animal. Human consumption of meat or other products from domestic animals can result in human ingestion of such insecticidal residues.

Accordingly, insect and pest control has been sought to be directed away from exclusive reliance on insecticides and towards the optimisation of environmental and economic insect and pest control (integrated pest management). The application of microbial control in which insects are attacked by pathogens such

as viruses, bacteria, fungi and protozoa are favoured as such microbial insecticides are highly selective for insect pests and do not leave toxic residues. However, microbial insecticides are not without their problems, such as the difficulty in applying them, as well as confining the natural enemy/parasite/disease to a large area. Further, they also have the disadvantages of short residual action and extreme specificity, which limit general applicability.

Genetic engineering has most recently been applied in the area of insecticides through the release of sterilised male insects and mass introduction of deleterious mutations such as chromosomal translocations. However, such procedures are very expensive, and stringent criteria are required before release of sterile males is contemplated. Chemosterilants which sterilise large segments of insect pest populations are also known, but these are strong carcinogens which precludes their use.

The search for new substances or approaches to pests and insect control has assumed increasing significance in public health in view of the use of chemical insecticides (and their environmental and economic viability), the nature and magnitude of the persisting residues, and increased insect and pest resistance, together with the toxicity levels of many synthetic chemical as well as natural chemical insecticides.

Of particular concern in the wool industry are the products used by woolgrowers to control or prevent lice and fly infestations. Currently, such products include synthetic pyrethroids, insect growth regulators and organophosphate products which cover nearly all the registered external parasitic treatments on small ruminants such as sheep and goats. All lice and fly products start to break down from the date

of application, but some of the chemicals remain in the fleece and tissue, as discussed above. This residual amount depends on the chemical used and the timing and rate of applications prior to shearing.

One specific problem in the wool industry is the late treatment of flystrike and lice in sheep before shearing. Sheep treated for flystrike and lice in long wool are a major source of high chemical residues on wool. To date, while the industry has sought measures such as introducing the lice and flystrike treatment early in the season and before 6 months before shearing, these measures are not often effective. Consequently, the search for a lice and flystrike prevention treatment which can be administered in the last 6 months of the growing season and which will produce wool with low chemical residues while still controlling lice and flystrike is a major commercial objective in the wool industry.

There is, therefore, a need for compounds which can be used as one or more active principles in insecticides, particularly in respect of insects including ectoparasites which afflict small ruminant economic animals, especially sheep and goats, and which are effective at low application rates, selective in biologic action and have low toxicity and a high margin of safety to humans, economic animals, aquatic organisms and birds. Such compounds should also be ones from which no persisting wool, tissue or other residues result, as well as being substances for which no insect or pest resistance exists. The substances must be environmentally friendly in that there must be demonstrably low impacts on the environment. They must also be economically viable to use on a large scale, particularly in respect of late season applications, that is applied within the last six months of the growing season.

Fermentation product A83543, also known as spinosyn, includes a family of related compounds (spinosyns) produced by *Saccharopolyspora spinosa*. These are naturally derived fermentation products with a positive safety profile in contrast to currently used synthetic organically derived compounds (such as synthetic pyrethroids, organophosphates, organochlorines and carbamates), and have previously been shown to exhibit insecticidal activity against insects affecting crops and transient activity against larval blow fly and adult stable fly when administered systemically to guinea pigs and sheep. By the terms "A83543 compounds" and "spinosyn and derivatives and analogues thereof" is meant components consisting of a 5,6,5-tricyclic ring system, fused to a 12-membered macrocyclic lactone, a neutral sugar [2N,3N,4N-(tri-O-methyl)rhamnose] and an amino sugar (forosamine), as described in the patents discussed *infra*.

The family of compounds from A83543 fermentation product has been shown to comprise individual compounds A83543A, A83453B, A83543C, A83453D, A83543E, A83453F, A83543G, A83453H, A83543J, A83453L, A83543M, A83453N, A83543Q, A83453R, A83543S, A83453T, A83453U, A83543V, A83453W, A83453X. Boeck *et al.* described spinosyns A-H and J and salts thereof in US patent Nos 5,362,634, 5,496,932 and 5,571,901. Mynderse *et al.* described spinosyns L-N, their N-demethyl derivatives and salts thereof in US patent No. 5,202,242. Turner *et al.* described spinosyns Q-T, their N-demethyl derivatives and salts thereof in US patent Nos 5,591,606, 5,631,155 and 5,767,253. Spinosyns K,O,P,U,V,W, and Y are described in the article by DeAmicis, C.V. *et al.* in American Chemical Society's Symposium Series: Phytochemicals for Pest Control

(1997), Chapter 11 "Physical and Biological Properties of Spinosyns: Novel Macrolide Pest-Control Agents from Fermentation," pp 146-154.

Spinosyn A (A83543A) was the first spinosyn isolated and identified from the fermentation broth of *Saccharapolyspora spinosa*. Subsequent examination of the fermentation broth revealed that the parent strain of *S.spinosa* produced a number of spinosyns (A83543A to H and J). Compared to spinosyn A, spinosyns B to H and J are characterised by differences in the substitution patterns on the amino group of the forosamine, at selected sites on the ring system and on the neutral sugar. The strains of *S.spinosa* produce a mixture of spinosyns, the primary components of which are spinosyn A (50-85%) and spinosyn D (15-50%). These are the two spinosyns that are currently known to be the most active as insecticides. The name "spinosad" refers to a mixture of spinosyn A and spinosyn D.

The present invention provides an insecticidal formulation for controlling an insect infestation in a small ruminant animal, said formulation comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent. The formulation may additionally comprise another insecticidal agent. In one embodiment, the amount of the spinosyn present in the formulation is such that the spinosyn residue in any wool, milk or tissue obtained from the animal immediately, or within about a month, following application of the formulation is at an environmentally acceptable level or safe to humans.

In these formulations, an effective amount of the spinosyn is present when the amount of spinosyn in the formulation is from 1-500 g of the spinosyn /L of the formulation. Preferably, the amount of spinosyn in the formulation is selected from the group consisting of 1-500, 1-400, 1-350 , 1-300, 1-250, 1-200, 1-150, 1-100,

1-90, 1-80, 1-70, 1-60, 1-50, 1-40, 1-30, , 1-25 or 1-20 g of spinosyn per L of the formulation. A preferred amount is 25 g/L.

In another aspect, this invention relates to an article of manufacture, comprising packaging material and a formulation for controlling an insect infestation in a small ruminant animal contained within said packaging material, wherein said formulation comprises

a topical unit dose of a formulation of this invention, i.e. one comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent; and

wherein said packaging material comprises a label or package insert with instructions for topically administering the dose to the animal.

This invention further relates to a use of a formulation of this invention, i.e. one comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent, in the preparation of a medicament for controlling an insect infestation in a small ruminant animal.

In another aspect, this invention provides the use of a formulation of this invention, i.e. one comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent, for controlling an ectoparasite infestation in a small ruminant animal selected from a sheep, goat, or camellid, comprising topically administering the formulation to the animal.

This invention further provides a method of controlling an ectoparasite infestation in a small ruminant selected from a sheep, goat or camellid, comprising topically administering a formulation of the invention, i.e., one comprising an effective amount of a spinosyn or an analogue or derivative thereof, to the ruminant.

When carrying out this method, it is preferable to administer a formulation wherein the amount of spinosyn in the formulation is such that the spinosyn residue in any wool, milk or tissue obtained from the animal immediately, or within about a month, following administration of the formulation is at an environmentally acceptable level.

Thus, the present invention provides formulations and methods of controlling, i.e. preventing, ameliorating or eliminating, ectoparasites, including insects and arachnids, in small ruminant animals such as sheep, goats and camellids by applying one or more A83543 compounds in an acceptable carrier or diluent.

Another aspect of this invention is a method for preventing, controlling and/or eliminating insects including ectoparasites in small ruminant animals by administering an insecticide to the animal, said insecticide including one or more spinosyn factors or analogues or derivatives thereof present

The invention also provides a method for controlling an ectoparasitic infestation, wherein the ectoparasite includes lice, fleas, ked, mites, itch mites, ticks and blowfly strike, in a small ruminant animal by administering to the animal, a spinosyn, or an analogue or derivative thereof, at a concentration of about 50 ppm or less, such that any wool, milk or tissue residue from the spinosyn in the animal is present in an environmentally acceptable amount immediately or within about a month following administration. In sheep, for example, an equivalent effect to a concentration of about 50 ppm of spinosyn is achieved by administering an amount of approximately 250 mg/head.

The invention further provides a method for controlling an ectoparasitic infestation, wherein the ectoparasite includes lice, ked and blowfly strike, in short wool sheep by administering to the sheep, a spinosyn, or an analogue

or derivative thereof at a concentration of from 1 to about 25 ppm, such that any wool, milk or tissue residue from the spinosyn in the sheep is present in an environmentally acceptable amount immediately or within about a month following administration.

The effect of a concentration of about 25 ppm of spinosyn can be achieved by administering about 125 mg/head to the sheep.

Other useful spinosyn concentrations for administering to short wool sheep are about 10, 3, and 1 ppm of spinosyn in the formulation.

In another aspect the invention provides a method for controlling an ectoparasitic infestation, including lice, ked and blowfly strike, in long wool sheep by administering to the sheep, a spinosyn, or an analogue or derivative thereof at a concentration of about 50 ppm or less, such that any wool, milk or tissue residue from the spinosyn in the sheep is present in an environmentally acceptable amount immediately or within about a month following administration.

Other useful spinosyn concentrations for administering to long wool sheep are about 25, 20, or 10 ppm.

Still another aspect of this invention provides a method for controlling an ectoparasitic infestation, including li e, ked and blowfly strike, in long wool sheep by administering to the sheep, a formulation wherein the spinosyn, or analogue or derivative thereof, is present at a concentration of about 50 ppm or less, such that any wool, milk or tissue residue from the formulation in the sheep is present in an environmentally acceptable amount immediately or within about a month following administration of the formulation.

Particularly useful methods for administering the formulations of this invention in short and long wool sheep are using a dip wash or jetting fluid.

Another aspect of this invention provides a method for controlling an ectoparasitic infestation, including lice, ked and blowfly strike, in long wool sheep by administering to the sheep in the last 6 months of the growing season, a spinosyn or an analogue or derivative thereof, at a concentration of about 50 ppm or less , such that any wool, milk or tissue residue from the spinosyn in the sheep is present in an environmentally acceptable amount.

For the purposes of the present application, the term 'insecticide' is defined to include acaracide, ectoparasiticide and miticides. Similarly, for the purposes of the present application, the term 'insect' is defined to include, but is not limited to mosquitoes, lice, fleas, flies, ticks and mites. The term 'ectoparasite' is similarly defined to include, but is not limited to, members of the insect order Diptera, Phthiraptera, and Acarina, and parasites and other insects which are parasitic during all of their life cycle or only part of their life cycle, such as only the larval or adult stage.

The term, 'small ruminant' or 'small ruminant animal' refers to sheep, goats or camellids (including alpacas, llamas and vacuna, etc.).

The term 'spinosyn or analogue or derivative thereof' is defined to include an individual spinosyn factor (A83543A-H, J-W or Y) an N-demethyl derivative of an individual spinosyn factor, or salt thereof, or a combination thereof. As stated above, the term "A83543 compound" is also used herein to mean an individual spinosyn factor, or a derivative or salt thereof, or a combination thereof.

The term "controlling an insect infestation" refers to preventing onset of an infestation of insects in a susceptible animal or to decreasing or eliminating the

number of living insects or viable insect eggs on the animal. The extent of reduction somewhat depends on the application rate of the compound and the compound used.

The term 'effective amount' means the amount which is sufficient to prevent or cause a measurable reduction in the insect population after treatment .

The term 'short wool sheep' means sheep with 6 weeks or less growth of wool since the last date of shearing.

The term 'long wool sheep' means sheep with more than 6 weeks growth of wool since the last date of shearing.

The term 'withholding period' means the period after treatment during which the wool/meat/milk of animals treated with a insecticide cannot be harvested. The withholding period of course differs in respect of the chemical nature of the active in the insecticide.

The term 'environmentally acceptable amount/level' refers to the amount of residue of an insecticide that is permissible in the wool, tissue and milk of treated small ruminant animals such as sheep, goats, and camellids. According to the Australian MRLs (Maximum Residue Levels) for spinosad, published in the Commonwealth of Australia Gazette (National Registration Authority for Agricultural and Veterinary Chemicals) or "NRA" January 1999 edn, edible offal (mammalian) has a MRL of 0.05 ppm (= 50 ng residue/g offal). Meat (mammalian in the fat) has an MRL of 0.2 ppm (=200 ng residue/g meat). In the U.S., the equivalent limits are: mammalian in the fat: 600 ng residue/g meat; edible offal: 200 ng residue/g offal and whole muscle: and 40 ng residue/g muscle.

In respect of wool residues, a recent report issued in November 1998 by the NRA and The Woolmark Company entitled *'The residue implications of sheep*

ectoparasiticides' addresses the 'environmentally acceptable amounts' of residues in sheep meat and wool in great detail. The current Australian guidelines state that the residue levels of insecticide on wool must not exceed 13 mg/kg raw wool and the residue levels in scouring effluent must not exceed 1 mg/L scouring effluent. The allowable amounts depend on the aquatic toxicity profile of the chemical. For the UK, synthetic pyrethroids (SPs), must be ≤ 0.06 mg/kg.

From the environmental toxicology the average allowable scouring lot concentration of spinosad is estimated to be approximately 15 mg/kg or higher.

An advantage of this invention is that the critical environmental residue limit for harvested wool and the relevant MRL in respect of meat and milk is met with a zero (0) withholding period in respect of meat/milk/tissue from sheep, goats and camellids treated with the formulations and methods of the present invention.

In other words, there is a zero withholding period in respect of meat/milk from small ruminant animals such as sheep, goats and camellids treated with the formulations and methods of the present invention, as well as a zero withholding period in respect of wool harvested from these animals treated with the formulations and methods of the present invention.

This invention is based on the surprising discovery in sheep that the spinosyns, i.e. a spinosyn factor or an analogue and derivative thereof, exhibit insecticidal activity at very low concentrations, much lower than those used or previously contemplated in sheep. It has been discovered that at even at 1 ppm, spinosyn compounds have 100% efficacy as insecticides, particularly

ectoparasiticides. Spinosyn compounds are usually in the form of either an emulsion or a suspension, but at such low concentrations they are advantageously in a solution form. As the spinosyn formulations of the invention are highly effective, they can be manufactured and supplied as low volume products which are easier to supply, take up less storage space and are easier to deliver to the farmer. The smaller quantities needed also means that the formulations can be supplied as dilute ready-to-use products in smaller packages which are easily disposed of.

Most importantly, these formulations and methods can be applied even late in the growing season (i.e., in the last 6 months). Due to the low concentrations at which the formulations are being used, they are practically non-toxic to humans and animals. Further, residues in the wool and tissue of animals treated with the formulations are reduced to environmentally acceptable levels. Minimal chemical residues are found in the milk of such animals treated with the formulations and methods of the present invention, which is of economic advantage to the farmer as processing costs are reduced. No skin irritation or dermal toxicity to shearers results from the methods and formulations of this invention. Environmental contamination is also minimised, with waste dip wash, waste jetting fluid and other run off from treated animals having minimal levels of chemicals and therefore representing only low environmental contamination.

Another advantage is that the spinosyns are very efficacious at low levels and have no apparent cross resistance to existing compounds. Thus, the present invention is of utility against parasite populations on sheep or small ruminants that have existing levels of resistance to currently used products.

As discussed *supra*, the formulations of the present invention can be used in controlling pests on small ruminant animals such as sheep, goats and camellids (including alpacas, llamas and vacuna etc.). Such pests include ectoparasites such as blowfly strike, lice, ked, mites, itch mite, sheep scab, screw worm, bot flies, ticks, fleas, and related arthropod pests. The spinosyns can be formulated into a variety of end use formulations and products which are easy to use.

In summary, food safety, operator safety and minimisation of adverse environmental effects are advantages resulting from the present invention. Thus, the present formulations provide advantages over commercially available ectoparasitic formulations for use in sheep and small ruminants.

Formulations containing the spinosyns are generally administered topically to the relevant animal. Such topical application can take the form of dipping, showering, jetting, spraying, manually applying such as dusting, or otherwise placing or laying the formulation containing the active substance/s on the animal. Accordingly, usually the spinosyn factor/s and analogues and derivatives thereof are formulated into a number of topically applied insecticidal formulations.

Topical insecticidal formulations include spot-ons, pour ons, sprays, dips, dusts, lotions, gels, ointments, salves, dressings, towels, cremes, sticks, soaps, shampoos, collars, medallions, eartags and tail bands. Pour-on formulations, including both aqueous and organic-solvent-based ones, as well as emulsions and suspensions, are preferred. More preferred are dip wash formulations, jetting fluid formulations and jetting/spray race formulations.

The active component-the spinosyn factor/compound- may be present as a single compound, a mixture of two or more compounds, a mixture including at least one of A83543A and A83543D, or a mixture of at least one A83543 compound together with the dried portion of the fermentation medium in which it is produced. One preferred active component used in the method of the present invention is spinosad which is a product comprised of 50-85% spinosyn A and 50-15% spinosyn D.

The active component-the spinosyn factor/compound- may also be present as a salt in the formulations and methods of this invention. The salts would be prepared using standard procedures for salt preparation. For example, spinosyn A can be neutralised with an appropriate acid to form an acid additional salt. The acid addition salts of spinosyns which can be used in the present invention are useful and include salts formed by reaction with either an organic or inorganic acid such as, for example, sulfuric, hydrochloric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, formic, lauric, stearic, salicyclic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic and other like acids.

The spinosyn factor/s or analogues or derivatives thereof can be mixed with one or more physiologically acceptable carriers or excipients to formulate the insecticide formulations of the present invention. The spinosyn factor/s or analogues or derivatives thereof can also be mixed with other commonly used ectoparasiticidal compounds in the insecticide formulations used in the present invention.

The formulations of the present invention can take the form of concentrated formulations which are dispersed in water for application, such as pour

on formulations, or dust or granular formulations which are applied without further treatment.

Preferably, the formulations of the active spinosyn compound are in the form of aqueous suspensions or emulsions prepared from concentrated formulations of the compounds. Such water soluble, water-suspendible or emulsifiable formulations are generally liquids, generally called emulsifiable concentrates or aqueous suspensions.

It is particularly preferable that the active formulations are in the form of a suspension concentrate. Emulsifiable concentrates and solution concentrates, as well as wettable powders, are also preferred.

Generally, emulsifiable concentrates of the A83543 compounds comprise a convenient concentration of an A83543 compound dissolved in an inert carrier which is either a water-miscible solvent or a mixture of a water immiscible organic solvent and emulsifiers. A preferred concentration range is 1-500 g/L of spinosyn compound. More preferably the concentration range is selected from the group consisting of 1-400 g/L, 1-350 g/L, 1-300 g/L, 1-250 g/L, 1-200 g/L, 1-150 g/L, 1-100 g/L, 1-90 g/L, 1-80 g/L, 1-70 g/L, 1-60 g/L, 1-50 g/L, 1-40 g/L, 1-30 g/L, 1-20 g/L, even more preferably 25 g/L. Useful organic solvents include aromatics including xylenes and petroleum fractions. Other organic solvents may also be used, such as the terpenic solvents, including rosin derivatives, aliphatic ketones such as cyclohexanone and complex alcohols such as 2-ethoxyethanol.

Suspension concentrates (SC) comprise suspensions of the active water-insoluble compound (including one or more spinosyn compounds) dispersed in an aqueous vehicle at a concentration in the range of from about 1-500 g/L.

Preferably, the concentration range is selected from the group consisting of about 1-400 g/L, about 1-300 g/L, about 1-250 g/L, about 1-200 g/L about 1-150 g/L, about 1-100 g/L, about 1-50 g/L, about 1-45 g/L, about 1-40 g/L, about 1-30 g/L, more preferably about 25 g/L. Generally the suspensions are prepared by finely grinding the spinosyn compound and mixing it into a vehicle comprised of water and surfactants chosen from such types as nonionic, sulfonated lignins and alkylsulfates and suspending agents such as xanthan and guar gums. Other inert ingredients may also be added.

The suspension concentrates and emulsions are preferably diluted with water to obtain the desired spinosad concentration in the final formulation which is applied in the methods of the invention. The active A83543 compounds can also be applied as insecticides to small ruminant animal species in the form of an aerosol composition. In such compositions, the active spinosyn compound is dissolved in an inert carrier, which is a propellant mixture.

Preferably, the carriers or excipients used in the /insecticidal formulations of the invention include dust carriers, solvents, emulsifiers, wetting and dispersing agents and water. Selection of the carrier is of course made on the basis of compatibility with the desired insecticide, including such considerations as pH, moisture content and stability.

In one preferred formulation, the insecticide takes the form of an emulsion comprising a solution of the active spinosyn compound/s in an organic solvent, with the optional addition of a surfactant. Water emulsion sprays from such an emulsion formulation are commonly used.

Other preferred formulations of the present invention can take the form of solids, e.g., wettable powders, dusts or granular compositions. These can then be compacted to form water dispersible granules. Wettable powders can be used as spray applications. Other possible formulations include granules or pellets where the carrier, such as an absorbtive clay, is impregnated with the active.

Advantageously, the method of the invention can be applied as an early season treatment, or as a late season treatment within the last 6 months of the growing season when the wool is long. The active spinosyn compound or compounds are generally present in a concentration of about 50 ppm or less upon application to sheep or other small ruminants. Other useful concentrations of the active spinosyn compound or compounds for application to sheep and other small ruminants are 35 ppm, 25 ppm, 20 ppm, 10 ppm, 5 ppm, 4 ppm, 3 ppm, 2 ppm, or 1 ppm.

Generally, the rate, timing and manner of effective application will vary with the identity of the parasite and other factors. In general, ectoparasite control is obtained with topical application of liquid formulations containing from about 1 to 10 ppm of spinosyn compound or compounds in respect of short wool animals and 10 to 50 ppm in respect of long wool animals. Conventional veterinary practices are used in application of the active compound to the animal. Such practices include spray, back rubbers, and dip tanks.

A preferred composition for use in the present methods of treatment is a suspension concentrate containing spinosad at a concentration of 25 g/L. If desired, a dispersant may be added to improve suspendability.

A preferred formulation contains the following ingredients:

Ingredient	Percent (w/w)	Sample batch (g)
Spinosad	2.76	41.4
Propylene glycol	10	150
Surfactant	2	30
Mineral Thickener	2	30
Xanthan Gum	0.2	3
Antimicrobial Agent	0.2	3
Antifoam Agent	0.1	1.5
Water, deionised	82.74	1241.1
Total	100	1500

In one embodiment of an aqueous suspension, the spinosyn is wet-milled as a "grind batch," i.e., spinosad, surfactant, water, and antifoam as needed. A typical "grind batch" contains the following ingredients:

	(g)
Spinosad	41.4
Surfactant	30
Water, deionised	100
Antifoam Agent	1
Total	**172.9**

A preferred nonionic surfactant to incorporate into the aqueous suspension of spinosad is PLURONIC P-123™.

A "hydrated suspension batch" is also formed by blending a hydrated suspension of the mineral thickener with xanthan gum hydrated in propylene glycol, along with an antimicrobial agent A typical hydrated suspension batch contains the following ingredients:

	(g)
Propylene glycol	150
Mineral Thickener	30
Xanthan Gum	3
Antimicrobial Agent	3
Water, deionised	1041.1
Total	**1227.1**

The hydrated suspension batch and additional water as needed are blended with the grind batch to prevent syneresis, or separation of clear watery fluid from suspended milled solids. The appropriate amount of the hydrated suspension batch to be blended with the grind batch to complete the formulation is determined based upon the percent recovery of the grind batch after particle size reduction.

A particularly useful mineral thickener is colloidal magnesium aluminum silicate, such as VEEGUM, that disperses and wells in water. A suitable xanthan gum in one that is heat stable with a good tolerance for strongly acidic and basic solutions such as RHODOPOL 23™.

Other formulations can also be used in the present methods of application of ectoparasiticidal formulations to sheep and small ruminants. For example, emulsifiable concentrates containing spinosad can be made and used for dilution and subsequent application to sheep. For example, a 25 g/L spinosad emulsifiable concentrate can be prepared in an aromatic hydrocadrbon such as Aromatic 150 containing the following ingredients: spinosad (90% active ingredient, 3.05 weight percent), sulfonate/nonionic surfactant blends (a total of 10 weight percent and aromatic hydrocarbon (86.9[illegible] weight percent). As an alternative, spinosad can be formulated as an emulsifiable concentrate in methyl oleate, potentially safer solvent, than the aromatic hydrocarbon.

The methods of the present invention, particularly the methods of treating ectoparasites in sheep can be performed in the following manner:

1) To eradicate lice, sheep should be throughly wetted to the skin, 2 to 6 weeks after shearing, with a solution containing 5 to 20 ppm spinosad. A plunge or

shower dip charged at about 5 to 20 ppm and reinforced at about 20 ppm is recommended.

2) To control lice in sheep with long wool (> 6 weeks after shearing), sheep should be hand jetted with about 25 ppm spinosad; 0.5 L should be used per month of wool per sheep. Plunge and shower dips and spray races may also be used.

3) To treat fly stike, the wound should be thoroughly wetted with about 25 ppm spinosad - applied as a wound dressing or using a jetting hand piece.

4) To prevent fly stike for around 4 (to 6) weeks, 25-50 ppm spinosad should be applied along the back, around the breech, around the pizzle of wethers and on the heads of rams; 0.5 L should be used per month of wool per sheep.

The methods as described above can also be used to control sucking lice, sheep ked and mites.

Examples

Example 1: Spinosad Dip Formulations for the Control of *Bovicola Ovis* on Sheep

Spinosad can be formulated as a suspension concentrate. Three formulations of spinsoad were made into two emulsifiable concentrates, one utilising an aromatic hydrocarbon solvent and one utilising oleate, and an aqueous suspension/suspension concentrate. Each formulation was prepared at two concentrations, namely containing 0.2 and 1 g/l spinosad. These were each diluted 1:5000 in water to give 0.04 and 0.2 ppm dip washes.

Six fine-wool Merino sheep infested with a strain of lice highly resistant to synthetic pyrethroids were plunge dipped in each dip two weeks after shearing.

Lice counts were conducted on the day of treatment before dipping and 7,14, 28, 42 and 56 days after treatment. The numbers of lice on sheep treated with water only (i.e. 0 ppm) increased slightly during the study.

The results with regards to lice numbers on sheep treated with the various formulations of spinosad in this example, are set out below in **Table 1.** **Subsections (a) - (g)** provide the lice counts observed with the control animals and with each concentration of the three listed spinosad-containing formulations.

Table 1. Lice Counts

a) **Control**

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
573	759	742	885	1128	1278	1371
1014	92	107	86	75	56	51
1020	147	123	87	101	98	90
1135	215	195	180	208	221	188
1189	147	100	110	190	170	128
1197	218	227	260	334	312	411
Geometric Mean	203.4	187.3	180.1	219.8	208.5	199.1
Arithmetic Mean	263	249	268	339.3	355.8	373.2

b) **Emulsifiable Concentrate in Aromatic Hydrocarbon at 0.04 ppm**

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
100	209	9	11	19	23	16
1003	147	41	41	76	121	124
1008	219	43	59	113	136	198
1021	503	77	68	137	164	229

1119	134	90	127	141	158	213
1132	95	46	63	84	75	78
Geometric Mean	187.2	41.4	49.4	80.1	95	106.9
Arithmetic Mean	217.8	51	61.5	95	112.8	143

c) Emulsifiable Concentrate in Aromatic Hydrocarbon at 0.2 ppm

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
1005	207	0	3	3	2	3
1010	147	14	14	11	10	13
1115	134	0	0	0	0	0
1120	113	4	10	1	5	3
1126	238	7	4	3	2	4
1187	493	82	53	74	121	157
Geometric Mean	194.5	5.1	6.5	4.5	5.5	6.5
Arithmetic Mean	222	17.8	14	15.3	23.3	30

d) Emulsifiable Concentrate in Methyl Oleate at 0.04 ppm

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
769	116	25	12	31	37	46
1013	134	38	38	59	67	58
1104	204	51	57	109	135	172
1124	244	104	107	163	141	242
1125	479	159	178	213	357	423
1136	148	47	49	71	116	71
Geometric Mean	194.9	57.9	53.8	88.8	111.8	122.2

Arithmetic Mean	220.8	70.7	73.5	107.7	142.2	168.7

e) Emulsifiable Concentrate in Methyl Oleate at 0.2 ppm

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
1004	133	2	6	4	5	2
1006	281	30	27	33	22	25
1110	338	2	4	2	3	2
1133	196	18	15	18	23	27
1139	116	8	3	17	17	21
2139	154	4	5	4	1	2
Geometric Mean	188.1	6.4	7.3	8.3	7.1	7
Arithmetic Mean	203	10.7	10	13	11.8	13.2

f) Aqueous Suspension at 0.04 ppm

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
1112	166	63	62	95	119	147
1137	323	166	95	146	249	268
1158	132	33	21	34	50	41
1177	122	62	53	86	213	203
1185	286	81	109	224	194	170
2084	195	65	71	129	183	291
Geometric Mean	190.7	69.5	60.8	102.7	149.6	159.1
Arithmetic Mean	204	78.3	68.5	119	168	186.7

g) Aqueous Suspension at 0.2 ppm

Sheep No.	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
809	126	1	4	6	8	4
1002	167	4	4	10	22	15
1085	289	26	6	17	49	50
1102	308	3	5	8	16	15
1163	187	42	40	38	42	60
2081	128	6	2	7	7	14
Geometric Mean	188.5	6.5	5.8	11.4	18.5	18.3
Arithmetic Mean	200.8	13.7	10.2	14.3	24	26.3

Example 2a: Dose determination for the treatment of sheep body lice (*Bovicola ovis*)

i) Short wool sheep, application < six (6) weeks after shearing in the form of a dip

Two dose titration studies were conducted in respect of spinosad applied as a dip for the control of *Bovicola ovis* on sheep. Spinosad, prepared as an emulsifiable concentrate, was diluted 1:1000 in water to obtain formulations of 0, 1.0, 5.0, 20 and 100 ppm concentration and formulations of 0, 0.04, 0.2 and 1.0 ppm concentration. Fine, short-wool Merino sheep infested with a strain of lice highly resistant to synthetic pyrethroids were plunge dipped in the dilute solution two (2) weeks after shearing. During the first titration study, 5 dips were made, with spinosad being applied at 0, 1.0, 5.0, 20 and 100 ppm to 6 sheep per each concentration by way of plunge dipping each group of six sheep. During the second titration study, spinosad was applied at four different concentrations: 0, 0.04, 0.2 and 1.0 ppm, again each concentration was applied to six (6) sheep by plunge dipping.

Lice counts were conducted on the day of treatment before dipping and 7, 14, 28, 42, 56 and 84 days after treatment. The number of lice on sheep treated with vehicle and water only (0 ppm) declined following dipping although remained at a satisfactory level for the duration of the trials. In the first titration study, a dose response was not observed as all spinosad treatments were 100% effective and no live lice were found after treatment.

In the second titration study, a dose response was observed. An average of 1.5 lice per sheep was seen 1 week after dipping in 1.0 ppm but on days 14, 28, 42, 59 and 84, 0-0.3 lice/sheep were seen. The 0.2 ppm formulation resulted in 73-94% efficacy and the 0.04 ppm dip gave 38 to 60% efficacy, depending on the day of counting. Based on the second study, the estimated concentration giving 100% efficacy was 2.0 ppm.

ii) Long wool sheep, application in the form of a jetting spray, 6 to 52 weeks after shearing

Thirty fine-wool Merino ewes with 6 months wool (5 cm long) were ranked and block allocated according to lice count into 5 groups of 6 sheep. Spinosad suspension concentrate (25 g/L) was diluted in water to give 0, 2, 5, 15 and 45 ppm dilutions. 5 L of each diluted concentrate was jetted onto six (6) sheep according to standard agricultural practice. The numbers of adult and nymphal lice at each of 40 fleece partings on each sheep was determined the day before jetting and 14, 28, 56 and 84 days after jetting. The control sheep (i.e., those treated with 0 ppm) were jetted with 350 ppm temephos 70 days into the study to relieve rubbing and skin irritation.

Spinosad applied at all rates provided >95% efficacy against sheep body lice within 14 days of treatment. At 56 days post treatment, spinosad at the

same rate of 45 ppm provided 100% efficacy and the 2.0 ppm rate gave 98.4% efficacy. The level of efficacy given by 2.0 ppm spinosad 14 days post treatment was greater than that provided by 350 ppm temephos 14 days post treatment.

Example 2b: Evaluation of spinosad for the treatment of blowfly strike on sheep caused by *Lucilia cuprina*

i) Fine wool sheep, application in the form of a wound dressing

Fifteen fine wool Merino sheep with approximately 6 months wool growth were implanted at six (6) sites with approximately 100 first instar of *Lucilia cuprina*. Forty eight hours after implantation, the sites were assessed and the viable sites treated. Emulsifiable concentrates containing 0, 2, 5, 15, 45 and 135 g/L spinosad were diluted 1: 1000 in water to give emulsions of 2, 5, 15, 45 and 135 ppm. Each treatment was applied to 3 sheep (18 sites) in 20, 40 or 60-mL volumes-2 volumes per sheep per concentration. Each site was examined for larval survival and development 24 hours after treatment.

As concentration increased, the treatments became progressively more lethal. For quick 100% efficacy, 80 ppm or more was required. Nonetheless, 15 and 20 ppm gave 100% efficacy at 83% of sites after 24 hours. All concentrations, however, had an adverse effect on larval survival and no strike in any treated site developed normally. Indeed, the surviving larvae seen in all the treated strikes in the first study would probably have died in the next 24 hours as was seen in the second study where all treatments gave 100% mortality at all sites 48 hours after application. Varying the volume of treatment applied from 20 to 60 mL had no effect on efficacy.

ii) Long wool sheep, application in the form of a jetting fluid

Ten cross bred Merino/Suffolk wethers with approximately 12 months wool growth and 2 Merino wethers with 6 months wool growth were implanted at 6 sites with approximately 100 first instar of *Lucilia cuprina*. Seventy two (72) hours after implantation, the sites were assessed and the sheep were treated. Suspension concentrate containing 25 g/L spinosad was diluted 1:1000 and 1:500 in water to give suspensions of 25 and 50 ppm. A 25 ppm suspension was also prepared with wetting agent added at the rate of 1:1000. Each treatment was applied to 4 sheep (24 sites) using a commercial jetting wand. Each site was examined for larval survival and development 1, 2.5-3 and 5-6 hours after treatment.

Within one hour of treatment at most sites, there was movement of larvae away from the site of implantation, up the wool staple. After 2.5 - 3 hours there were no normal larvae (except 1 site that jetting fluid had not reached) and many abnormal larvae. By 5-6 hours after treatment, most larvae were dead or missing from the site of implantation. Increasing concentration from 25 to 50 ppm did not improve efficacy and neither did adding surfactant.

iii) Prevention. evaluation of spinosad for the prevention of blowfly strike on sheep caused by *Lucilia cuprina*

Spinosad emulsifiable concentrates were diluted 1:1000 in water to give 0, 2, 5, 15 and 45 ppm emulsions and applied as jetting fluids to the backs of each of 3 sheep. Fifteen fine wool merino sheep with approximately 6 months wool growth were used. 3, 8, 15, 22, 29 and 36 days after treatment each sheep was implanted at two sites on the back with approximately 100 first instar *Lucilia cuprina* maggots. Each site was examined for maggot survival and development 24, 48 and 72 hours after implantation. The degree of maggot killing or inhibition of maggot

development was related to concentration and time since treatment. 2 ppm gave 100% mortality of applied maggots at 3 or more of 6 sites for less than 2 weeks. Increasing the concentration to 5 ppm extended maggot death at 3 of the 6 sites to 4 weeks. The 15 ppm results were variable, but only lasted 4 weeks. 45 ppm gave complete mortality of maggots at 4 of 6 or 5 of 6 sites for 4 weeks and at 3 of 6 sites at 5 weeks.

Example 2c: Evaluation of spinosad for the treatment of sheep ked (*M. ovinus*)

i) Three (3) shorn sheep and 3 unshorn sheep sprayed with 1 L each of 100 ppm spinosad.

Shorn sheep - 100% keds dead by 1 day after treatment

Unshorn sheep- some live ked seen 1, 3 and 7 days after treatment. All were dead by 14 days after treatment.

ii) Six (6) recently shorn sheep infested with ked were divided in 3 groups of 2 sheep. The sheep in group 1 were sprayed with 1 L containing 25 ppm spinosad; those in group 2 with 50 ppm spinosad, and the group 3 sheep were treated with vehicle only (controls). Sprays containing 25 and 50 ppm spinosad killed all keds within 1 day. Counts on control sheep declined after shearing but much less rapidly than treated sheep.

Example 3a: Dose confirmation and protection period for the treatment of sheep body lice (*Bovicola ovis*)

i) Short wool sheep, application < six (6) weeks after shearing in the form of a dip

Thirty-five lice-free Merino sheep were plunge dipped in an aqueous suspension containing 5 ppm spinosad. 35 similar lice-free sheep were used as controls. 1, 2, 3, 4 and 6 weeks after treatment, five (5) treated and 5 untreated (control) sheep were implanted with 50 viable lice on the shoulder and hip. The implantation sites were examined 4 weeks later and again another 4 weeks later if no live lice were found at the first examination.

Lice placed on treated sheep 1 week after treatment failed to survive. The lice placed on the untreated sheep survived. Lice implanted 2 weeks after treatment survived in low numbers on 2 of 5 treated sheep and on all of the 5 control sheep. Numbers of surviving lice increased with time between dipping and times of subsequent examination. By five (5) weeks after treatment, lice numbers on treated sheep were approaching those seen on untreated control sheep.

Example 4: Decay profile of spinosad in sheep wool following dipping

Spinosad suspension at 5 g/L and 20 g/L was diluted 1:1000 with water. The concentration of the two dip washes were therefore 5 ppm and 20 ppm, respectively. Nine Merino ewes with 2 weeks wool and having had no ectoparasite treatment for 12 months were used and divided into 3 groups of 3 sheep. Each group was housed in an outdoor pen, which was raised with a mesh floor. Each pen had a roof over 1/3 of the pen and had metal walls between each pen.

Two sheep dip washes were prepared in a calibrated stainless steel bath. 475 mL of dam water was added to the bath, 475 mL of test article was then added to the bath and mixed thoroughly and a 40-mL sample of the dip was collected.

6 of the sheep were individually dipped for 30 seconds with the head submerged twice in the 5 ppm dip wash, while 3 sheep were similarly individually treated in the 20 ppm dip wash.

Following dipping, circumferential wool samples were collected using electric hair clippers with a cutting width of 4.5 cm from all sheep 3 days prior to treatment and on a range of days post treatment. Three of the sheep treated with 5 ppm spinosad were sampled on days 1, 2, 4, 7, 9, 11, 14, 17 and 21 post treatment, while the remaining three sheep were sampled on days 7, 14, 21, 28, 35, 42, 49, 56 and 64. The three sheep treated with 20 ppm spinosad were sampled on days 7,14, 21, 28, 42, 56, 70, 86 and 98. The wool samples were then analysed by immunoassay.

Following dipping in 5 ppm spinosad, there was a maximum residue of 3.3 ppm which declined with a half life of about 2 weeks. After 64 days, the residue was 0.27 ppm which was 8% of the initial residue. Following dipping in 20 ppm, a residue of 16.4 ppm was seen, which took 5 weeks to decline to 8 ppm, but after that the half life was about 2 weeks. After 98 days, the residue was 1.36 ppm, which was 8% of the initial residue.

Example 5: Spinosad residues in sheep meat, fat and offal four days following dipping in 50 ppm spinosad dip formulation

Two previously sheared sheep were dipped in an aqueous suspension dip formulation containing 50 ppm spinosad for 30 seconds, with the head being dunked twice, and then placed in a floor pen for four days. The sheep were sacrificed and necropsied at the end of those four days; and samples of kidney, liver, muscle, perirenal and abdominal fat were collected. The tissues were transferred to a -20°C freezer until processing. The tissue samples were chopped, frozen with dry ice and

then ground using a hammermill. The samples were then placed in a -20°C freezer until assay. Samples were assayed using Elanco Method B05873 (EPA Method MRID 44058822, DowAgroScience Method GRM 95.03) "*Determination of spinosad and metabolites in Beef tissues, milk and cream by HPLC with UV detection*" which is certified for use with sheep tissues. The samples were assayed in duplicate with the results shown below in **Table 2**. The residue definition of spinosad is the sum of spinosyn A and spinosyn D. However, all residues are spinosyn A, as residues of spinosyn D, N-demethyl spinosyn D and spinosyn B were less than the limit of quantitation of the certified method.

Table 2: Total Spinosad Residue (ng/g) - Day 4 after 50 ppm dipping

	Animal A	Animal B	Average
Abdominal fat	150.0	177.5	163.8
Perirenal fat	99.9	142.7	121.3
Liver	34.2	38.9	36.6
Kidney	23.3	28.3	25.8
Muscle	14.9	13.5	14.2

Example 6: Measurement of the residue decay profile of spinosad in sheep wool following dipping in 10 ppm spinosad and jetting with 25 ppm spinosad.

Table 3. Mean concentration (mg/kg) and total mass (µg) of spinosad on wool band samples collected after sheep were dipped in 10 ppm spinosad suspension in water at days 1 through to 170 after treatment.

Days	1	7	14	28	42	58	84	113	140	170
Concentration	10.503	4.803	5.844	2.001	1.034	0.441	0.184	0.114	0.066	0.022
Mass	371.35	163.63	275.61	124.51	80.35	35.49	19.09	11.38	9.31	3.20

Merino sheep with 6 weeks wool growth were dipped in an aqueous suspension containing 10 ppm spinosad. Circumferential strips of wool were collected from each sheep after treatment to allow the estimation of spinosad residues and residue decay profiles. Wool residues decayed with an average degradation rate (half-life) of 26 days.

Table 4. Mean concentration (mg/kg) and total mass (μg) of spinosad on wool band samples collected after sheep were jetted with an aqueous suspension containing 25 ppm spinosad at days 1 through to 84 after treatment.

Days	1	7	14	28	42	58	84
Conc	7.63	5.13	5.19	6.13	3.39	1.28	0.69
Mass	1156.4	759.9	835.4	990.6	641.0	250.1	154.4

Spinosad decayed with a half-life of 30 days in sheep with 9 months wool growth jetted with 25 ppm spinosad.

On the basis of the toxicity of spinosad to aquatic organisms, a provisional environmental assessment of discharges from wool scouring has established target concentrations for wool processing lots in Australia and in Europe of 66 mg/kg and 15 mg/kg respectively. (I M Russell, N T Campbell, J T Rothwell, *Proc Aust Sheep Vet Soc 2000*, pp 108-115, AVA Conf Perth 2000). The wool residues seen immediately following treatment are well below the projected maximum permissible residues for scouring lots in Europe which has the most stringent Environmental Quality Standards. Even following late season jetting treatment for blowfly prevention, sheep will be left at least a month before shearing and following other treatments for up to 12 months after treatment allowing wool residues to decay. In addition the wool in a processing lot is made up of wools treated in early season, wools treated in late season, untreated wools, and wools treated with other chemicals. This blending will allow the spinosad residue from any farm to be diluted by these other wools. Therefore treatment of sheep with spinosad at the recommended rates will not cause wool residues to breach any wool scour environmental parameters and will not require the establishment of a wool with-holding period following treatment.

Spinosad has a high margin of safety for farm workers due to the short half-life of spinosad in wool grease, the very low level of dermal absorption and low mammalian toxicity of the active ingredient. Sheep treated with spinosad present no risk to shearers, wool handlers or other farm workers.

Example 7: Tissue residues following dipping in 10 ppm spinosad and jetting with 25 ppm spinosad.

Thirty seven fine wool Merino ewes and wethers with 6 weeks wool growth were dipped in 10 ppm spinosad suspension in water. Thirty seven Merinos with 9 months wool growth were jetted with 25 ppm spinosad. Animals were slaughtered 5, 12, 15, 21, 39and 56 days after treatment and samples of back fat, muscle, liver, peri-renal fat and kidney collected and assayed for spinosad residue. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.003 mg/kg. Residues of spinosad greater than the limit of quantification were found in fat samples from animals treated via a plunge dip and traces (values between the LOQ and LOD) were found in fat from jetted animals. Traces of spinosad were found in the liver and kidney tissues of animals that were plunge dipped, but except for one liver sample collected 12 days after treatment, not from jetted animals. Offal residues were below the LOD 15 days after treatment. Residues of spinosad in the main edible tissue of sheep, meat were undetectable at all times.

The highest residues recorded were in tissues collected 5 days after dipping and were 3 to 5 times lower than the lowest relevant residue limit set. Therefore dipping sheep in 10 ppm spinosad or jetting them with 25 ppm spinosad does not infringe established minimum residue limits (MRL) or tolerances and no with-holding period is required. In countries were a MRL is not established and a nil residue is required a withholding period of 56 days would ensure that the tissue with the highest residue - fat - would be below the LOD. Likewise offal would be below the LOD 15 days after treatment and meat residues would be below the LOD at all times after treatment.

Tables 5. Mean spinosad residues (mg/kg) in fat after dipping in 10 ppm spinosad.

Days after treatment	5	12	15	21	39	56
Peri-renal fat	31	18	26	24	10	2
Back fat	15	20	21	16	13	0

CLAIMS:

1. An insecticidal formulation for controlling an insect infestation in a small ruminant animal, said formulation comprising an effective amount of a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent.

2. A formulation of **Claim 1** wherein the spinosyn is a component of spinosad.

3. A formulation of **Claim 1** or **2** wherein the amount of spinosyn in the formulation is selected from the group consisting of 1-500, 1-400, 1-350 , 1-300, 1-250, 1-200, 1-150, 1-100, 1-90, 1-80, 1-70, 1-60, 1-50, 1-40, 1-30, , 1-25 or 1-20 g of spinosyn per L of the formulation.

4. A formulation of **Claim 1, 2** or **3** wherein the ruminant animal is a sheep, a goat or a camellid.

5. A formulation of **Claim 1, 2, 3** or **4** wherein the amount of spinosyn present in the formulation is such that the spinosyn residue in any wool, milk or tissue obtained from the animal immediately, or within about a month, following application of the formulation is at an environmentally acceptable level.

6. A formulation of **Claim 1, 2, 3, 4,** or **5** which additionally comprises another insecticidal agent.

7. An article of manufacture, comprising packaging material and a formulation for controlling an insect infestation in a small ruminant animal contained within said packaging material, wherein said formulation comprises

a topical unit dose of a formulation of **Claim 1, 2, 3, 4, 5** or **6**; and wherein said packaging material comprises a label or package insert with instructions for topically administering the dose to the animal.

8. The use of a formulation of **Claim 1, 2, 3, 4, 5** or **6** in the preparation of a medicament for controlling an insect infestation in a small ruminant animal.

9. A use of a formulation of any one of **Claims 1 to 6** for controlling an ectoparasite infestation in a small ruminant animal selected from a sheep, goat, or camellid, comprising topically administering the formulation to the animal.

10. A method of controlling an ectoparasite infestation in a small ruminant animal selected from a sheep, goat, or camellid, comprising topically administering a formulation of any one of **Claims 1 to 6** to the animal.

11. A method of **Claim 10** wherein the animal is a sheep, and the sheep is a short wool sheep.

12. A method of **Claim 11** wherein the effective amount of the spinosyn in the formulation is 10 ppm or less.

13. A method of **Claim 12** wherein the formulation is administered in a dip wash or jetting fluid.

14. A method of **Claim 13** wherein the effective amount of the spinosyn in the formulation is 5 ppm or less.

15. A method of **Claim 14** wherein the amount is 3 ppm or less.

16. A method of **Claim 15** wherein the amount is about 1 ppm.

17. A method of **Claim 10** wherein the animal is a sheep, and the sheep is a long wool sheep.

18. A method of **Claim 17** wherein the effective amount of the spinosyn in the formulation is 50 ppm or less.

19. A method of **Claim 18** wherein the formulation is administered in a dip wash or jetting fluid.

20. A method of **Claim 19** wherein the effective amount of the spinosyn in the formulation is 25 ppm or less.

21. A method of **Claim 20** wherein the amount is 20 ppm or less.

22. A method of **Claim 21** wherein the amount is 10 ppm or less.

23. A method of **Claim 17** wherein the formulation is administered within the last six months of the season.

24. A method of **Claim 10** wherein the ruminant is a goat.

25. A method of **Claim 10** wherein the ruminant is a camellid.

26. An insecticidal formulation for controlling an insect infestation in a small ruminant animal, said formulation comprising a spinosyn, or an analogue or derivative thereof, together with an acceptable carrier or diluent, substantially as hereinbefore described with reference to any one of the Examples.

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 00/17869

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N43/22 //(A01N43/22,25:34,25:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	---	1-26
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2000	19/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lamers, W

Form PCT/ISA/210 (second sheet) (July 1992)

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Intern: al Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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